Iron Chelation With Desferrioxamine B in Adults With Asymptomatic Plasmodium falciparum Parasitemia

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To determine if iron chelation therapy has activity against human malaria, we administered desferrioxamine B in amounts of 100 mg/kg per day by continuous 72-hour subcutaneous infusions to 28 volunteers with asymptomatic Plasmodium falciparum infection in a randomized, double-blind, placebo-controlled crossover trial. Peripheral blood concentrations of P falciparum ring forms were determined at 12-hour intervals in all subjects and serum concentrations of desferrioxamine B + ferrioxamine (the iron complex of desferrioxamine B) were measured in 28 subjects. Geometric mean concentrations of asexual intraerythrocytic parasites decreased with both chelator and placebo treatment, but the decrement with desferrioxamine B was significantly greater than that with placebo (P < .006) during both the initial and crossover periods. Compared with placebo, desferrioxamine B treatment was associated with an almost 10-fold enhancement of the rate of parasite clearance during both phases of the trial (P < .007). Mean ± SEM steady state concentrations of desferrioxamine B + ferrioxamine were 6.90 ± 0.60 μmol/L at 36 hours and 7.72 ± 0.68 μmol/L at 72 hours; in vitro, the ID₅₀ has been reported to be approximately 4 to 20 μmol/L. No drug toxicity was detected. Parasitemia recurred in 19 of 24 participants followed-up over 1 to 6 months. We conclude that desferrioxamine B enhances the clearance of P falciparum parasitemia and that iron chelation may provide a new strategy to be developed for the treatment of malaria.

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Several iron-chelating agents are capable of inhibiting the growth of Plasmodium falciparum in vitro. Iron is required for a number of essential enzyme systems operative in the erythrocytic trophozoite, including dihydroorotate dehydrogenase for the synthesis of pyrimidines, phosphoenol pyruvate carboxykinase in CO₂ fixation, and cytochrome oxidase of the mitochondrial electron transport system. In addition, the iron-dependent enzyme ribonucleotide reductase has been considered a potential site of action for an iron-chelating agent. The pathogenesis of malaria is a function, in part, of the ability of the intraerythrocytic parasite to reproduce at an extremely rapid rate, requiring a high rate of trophozoite DNA synthesis. Ribonucleotide reductase catalyzes the reduction of CDP, UDP, GDP, and ADP to their corresponding deoxynucleotides, steps that are rate limiting for DNA synthesis. In vitro, iron chelation can reversibly inhibit ribonucleotide reductase and produce a potent inhibition of DNA synthesis in a variety of cell systems.

Desferrioxamine B, a naturally occurring trihydroxamic acid derived from cultures of Streptomyces pilosus, is the only drug currently available for clinical use as an iron chelator. A generally safe and nontoxic agent, it must be administered by continuous parenteral infusion to be effective; daily doses of up to 150 mg/kg are used for therapy of iron overload. Preclinical data suggested that desferrioxamine B might have an antimalarial effect in humans: (1) a mean plasma concentration of 20 μmol/L was achieved when desferrioxamine B was administered by continuous parenteral infusion to seven non-iron-loaded adults at 100 mg/kg/d, (2) the growth of P falciparum in cultured erythrocytes was suppressed when desferrioxamine B in concentrations of 2 to 20 μmol/L was added to the medium, and (3) parenteral administration of desferrioxamine B in doses of 60 to 125 mg/kg/d inhibited parasitemia with P vinckei and P berghei in rodents and P falciparum in Aotus monkeys. Desferrioxamine B is apparently able to enter parasitized red cells and the available evidence suggests that the chelator exerts its antiparasitic effect by binding intracellular parasite-associated iron and making it unavailable to essential plasmodial enzymes. To determine if iron-chelation therapy with desferrioxamine B has activity against human infection with P falciparum, we administered subcutaneous infusions of the drug to partially immune adults with asymptomatic parasitemia.

MATERIALS AND METHODS

Study design. The study was approved by the Ethical and Research Committee of the University of Zambia and by the Committee on Human Investigation of MetroHealth Medical Center, Case Western Reserve University. The study was conducted from February to August 1990, encompassing both months when malaria transmission is high and when it is low. Informed consent was obtained from all participants. Partially immune subjects with asymptomatic parasitemia were identified by obtaining thick blood smears from healthy, nonpregnant, and nonlactating community members, staining with Giemsa, and examining for the presence of P falciparum ring forms. In this endemic area, subjects with asymptomatic parasitemia probably include (1) individuals with a low but steady level of parasitemia as a result of...
long-standing immune clearance and (2) persons with a subclinical episodic infection. Study preparations (desferrioxamine B or placebo) were administered by continuous subcutaneous infusions via portable, battery-operated pumps (Cormed ML-6-4; Conming, NY) and Medfusion 300 (Medfusion Systems, Duluth, GA). Desferrioxamine B (Desferal) was donated by Ciba-Geigy, Ltd (Basel, Switzerland). Placebo solutions consisting of normal saline were prepared by the pharmacy of Macha Hospital.

Twenty-eight individuals were entered into a randomized, double-blind, crossover trial comparing desferrioxamine B (100 mg/kg/d) and placebo; subjects included 13 men and 15 women with ages ranging from 15 to 56 years, median 18 years. The participants received subcutaneous infusions for 6 days: desferrioxamine B and placebo were each administered for 72 hours with the sequence of administration determined by random assignment. At the end of each 72-hour period, any remaining solution was measured. Finger puncture blood samples were obtained at approximately 12-hour intervals to prepare thick blood films for the quantification of ring forms. Venous blood was collected in tubes containing potassium-EDTA at 72-hour intervals to determine blood counts. Serum samples were obtained at 36-hour intervals and frozen within 12 hours at −5°C to −10°C for later determinations of concentration of the sum of desferrioxamine B and ferrioxamine (the iron complex of desferrioxamine B).

The patients were examined and questioned regarding side effects twice daily. Although auditory and visual neurotoxicities have been described in patients receiving long-term desferrioxamine B therapy for iron overload,10 facilities for performing audiometric assessment and thorough fundoscopic evaluation were not available in the villages where the study was conducted. The short duration of therapy in this study would probably lessen the risk for such complications.

Laboratory determinations. Laboratory determinations were performed by personnel other than those administering the experimental therapy to the volunteers. White blood cells (WBCs) were quantified with a counting chamber. The concentrations of peripheral blood asexual erythrocytic parasites were estimated by a standard method.11 Briefly, a thick blood smear was stained with Giemsa and the number of ring forms per 200 WBCs was counted and multiplied by the most recent WBC count (WBCs/µL). If there were less than 10 parasites per 200 WBCs, then the number per 500 WBCs was determined. The sum of desferrioxamine B and ferrioxamine was assayed using a liquid chromatographic method (S. Hauffe, Ciba-Geigy Ltd, personal communication, 1990). The method was validated on each day of analysis by analyzing serum samples spiked with desferrioxamine B or ferrioxamine or both. The limit of detection was about 0.03 µmol/L. During storage, some desferrioxamine B may complex with iron to form ferrioxamine, so this analytic method cannot reliably distinguish between the two forms of the chelator in stored serum samples. Because the subjects in this study probably had normal or decreased amounts of body iron, the sum of desferrioxamine B plus ferrioxamine represents mainly desferrioxamine B.

Statistical analysis. Parasite counts were found to have a log normal distribution and were transformed logarithmically for analysis. Proportional declines in log parasite concentrations were determined by subtracting values at time 0 from those determined at 12, 24, 36, 48, 60, and 72 hours. The Student's t-test for independent samples was used to compare geometric mean parasite concentrations at time 0 in the crossover trial participants randomized to receive desferrioxamine B initially versus those given placebo first. The paired t-test was used to compare steady state concentrations of desferrioxamine B plus ferrioxamine at 36 and 72 hours. Repeated measures analysis of variance was used to compare log parasite counts and proportional declines in parasite concentrations (1) within each treatment arm during infusions of desferrioxamine B and placebo and (2) between the two treatment arms. The χ² test was used to compare the prevalences of side effects during infusions of desferrioxamine B and placebo.

RESULTS

Crossover trial. All 28 volunteer participants in the placebo-controlled crossover trial received a study preparation for the first 72-hour period, but three did not continue for the second 72-hour infusion. Two of these volunteers declined further participation because of discomfort at the infusion site and inconvenience in wearing the pump. The third individual was removed from the study because she developed mild headache and low-grade fever that was initially presumed to represent clinical malaria. She immediately was treated empirically with chloroquine, but subsequently the parasite count was found to be 0 at the time of these symptoms and her illness was considered likely to have been of viral origin. An infusion pump malfunctioned on one occasion and the subject using the pump was changed to intravenous infusion of part of the placebo infusion and all of the desferrioxamine B solution. Another volunteer who was scheduled to use the defective pump was instead administered both desferrioxamine B and placebo intravenously. Among the 27 participants who received desferrioxamine B, the mean (±SEM) amount of the chelator that was actually infused over 72 hours was 290 ± 4 mg/kg, or 97% of the planned quantity of 300 mg/kg.

For the three participants who completed only the initial phase of the crossover trial, peripheral blood asexual parasites decreased from 600/µL to 0/µL and from 300/µL to 0/µL in the subjects who received desferrioxamine B, and from 185/µL to 110/µL in the individual who received placebo.

Geometric mean concentrations of peripheral blood parasites over two 72-hour treatment periods for the 25 individuals who completed the crossover trial are shown in Fig 1. At time 0, geometric mean peripheral blood parasite concentrations were similar in the 12 subjects who received desferrioxamine B first (1,148 rings/µL; SE range of 776 to 1,698) and the 13 subjects who were administered placebo first (1,319 rings/µL; SE range of 832 to 2,089) (P was not significant). Geometric mean concentrations of ring forms decreased throughout the crossover trial with both treatments. Decreases with placebo were significant during the initial phase (P = .002) and approached but did not reach significance during the crossover phase (P = .06), while declines with desferrioxamine were significant during both phases (P = .0001 for each period). Notably, when the magnitudes of the declines in mean parasite concentrations during desferrioxamine B therapy were compared with those with placebo, the decrements with desferrioxamine B were found to be significantly greater both in the initial (P = .005) and crossover (P = .0001) periods. The rates of parasite clearance during the crossover trial are shown in Fig 2 as the proportional decreases in parasite concentration over the two 72-hour periods of study, expressed logarithmically. Compared with placebo, desferrioxamine B treatment was associated with an almost 10-fold enhance-
Fig 1. Concentrations of *P lacticum* ring forms in 25 partially immune subjects with asymptomatic parasitemia who completed a randomized, double-blind, crossover trial comparing desferrioxamine B (100 mg/kg/d) and placebo administered by 72-hour subcutaneous infusions. Values are mean ± SEM. (B) Desferrioxamine B administered in initial treatment period; (C) placebo administered in initial treatment period. Statistical comparisons in initial treatment period: change in mean parasite concentrations with desferrioxamine B, \(P = .0001\); change with placebo, \(P = .002\); change with desferrioxamine B compared with that with placebo, \(P = .005\). Statistical comparisons in the crossover treatment period: change in mean parasite concentrations with desferrioxamine B, \(P = .0001\); change with placebo, \(P = \text{not significant}\); change with desferrioxamine B compared with that with placebo, \(P = .0001\).

Discussion

Our study was designed to determine if iron chelation is potentially useful in the treatment of human malaria. The emergence of resistance to all of the antimalarials currently in use provides the rationale for evaluating new therapeutic strategies. As a World Health Organization panel noted recently, “Most of the standard antimalarial drugs have been in use for 30 years or more and the increasing problem of drug resistance and the failure to reduce the transmission of malaria in many regions have emphasized the limitations of these drugs and made the search for new and more effective compounds imperative.” In the present study we attempted to reduce parasitemia in asymptomatic adults by exploiting the dependence of the malarial parasite on the essential nutrient, iron.

We used the only iron-chelating agent now approved for clinical use, desferrioxamine B, recognizing that this drug would be unlikely to find widespread clinical application because of its expense and the requirement for parenteral administration. When administered orally, desferrioxamine B is poorly absorbed. To be effective, the drug must be administered by continuous subcutaneous or intravenous infusion. In the dose and duration used in this study, desferrioxamine B could lead at most to the excretion of a few milligrams of iron in a person with normal or reduced iron stores, a quantity that would not substantially affect a patient’s iron content. Furthermore, a recent study suggests that malaria infection in humans is not influenced by iron status, and it has been shown that the antimalarial action

desferrioxamine B a median of 4 months after participating in the study (range, 1 to 6 months). Thick smears were positive in 19 (79%) of these individuals even though they were asymptomatic.

Serum concentrations of desferrioxamine B. Determinations were performed on serum samples obtained at 36 hours and 72 hours after beginning the infusion of desferrioxamine B in 26 subjects. Mean (±SEM) steady state concentrations of desferrioxamine B + ferrioxamine were 6.90 ± 0.60 μmol/L at 36 hours and 7.72 ± 0.62 μmol/L at 72 hours; these means are not significantly different.
of desferrioxamine B in laboratory animals is independent of host iron status.\textsuperscript{22} We did not include a control period with the administration of ferroxamine (desferrioxamine B bound to iron) in our experimental design, but note that ferroxamine has been examined in patients admitted to hospital with clinical malaria, we chose the duration of the study. For this initial trial, we chose 72 hours as the minimum period of treatment that might be expected to show activity of the drug. While anticipating that a single 72-hour course of desferrioxamine B would permit detection of an antimalarial effect, this duration of therapy was considered unlikely to result in the complete elimination of the parasite. Studies in vitro have provided evidence that desferrioxamine B is cytocidal against \textit{P. falciparum}, but suggested that the chelator acts specifically at the late trophozoite stage of the intraerythrocytic parasite.\textsuperscript{10,23} The intraerythrocytic life cycle of \textit{P. falciparum} lasts for about 48 hours,\textsuperscript{24} but is not well synchronized in contrast to other species of malaria; the life cycle in ‘laggards’ may last considerably longer.\textsuperscript{25} A chelator dose of 100 mg/kg/d produces serum concentrations in the range of the reported ID\textsubscript{90} for \textit{P. falciparum} in vitro, but might not consistently achieve levels that lead to 100\% inhibition of the parasite. Thus, a single 72-hour subcutaneous infusion of desferrioxamine B (100 mg/kg/d) would be unlikely to expose all parasites to lethal concentrations of the chelator at the susceptible stage of parasite development.

Given the restrictions of our experimental design, the results of the double-blind, placebo-controlled trial of desferrioxamine B provide unequivocal evidence that this iron-chelating agent has antimalarial activity against human infection. As shown graphically in Figs 1 and 2, geometric mean concentrations of \textit{P. falciparum} asexual forms decreased throughout the crossover trial with both treatments. The overall mean decline during placebo periods presumably resulted from immune clearance of the parasites. With desferrioxamine B, the decrement in geometric mean parasite concentrations was almost 10-fold greater than that found with placebo therapy during both the initial and crossover periods. Thus, the iron-chelating agent seemed to exert a potent antimalarial effect, with complete clearance of \textit{P. falciparum} parasitemia in more than 90\% of those treated. The steady state mean serum concentrations (7 to 8 \textmu mol/L) of desferrioxamine B achieved with predominantly subcutaneous administration of the drug in doses of 100 mg/kg/d in this study were at the lower end of the inhibitory range of 2 to 20 \textmu mol/L and the range of values reported for the ID\textsubscript{90} for 4 to 20 \textmu mol/L, as determined in vitro.\textsuperscript{15,19,20} We thank Dr Simeon Pollack for his suggestion to compare desferrioxamine B with placebo in subjects with asymptomatic parasitemia, Dr C. Schindler and Dr A. Poltera of Ciba-Geigy for their helpful advice, and Dr Laura Holmes, Dr Elizabeth McKinley, and Dr R.C. Gilkeson for their help in preliminary studies.

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REFERENCES


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Iron chelation with desferrioxamine B in adults with asymptomatic Plasmodium falciparum parasitemia [see comments]

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